Design and synthesis of paclitaxel-containing aminoester phosphate and phosphoamidate

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Paclitaxel-containing aminoester phosphate 4 and phosphoamidate 9 were synthesised and found to possess anticancer activity against HL-60 leukemia cells; their solubility in a phosphate buffer solution was about 16 times higher than that of paclitaxel.

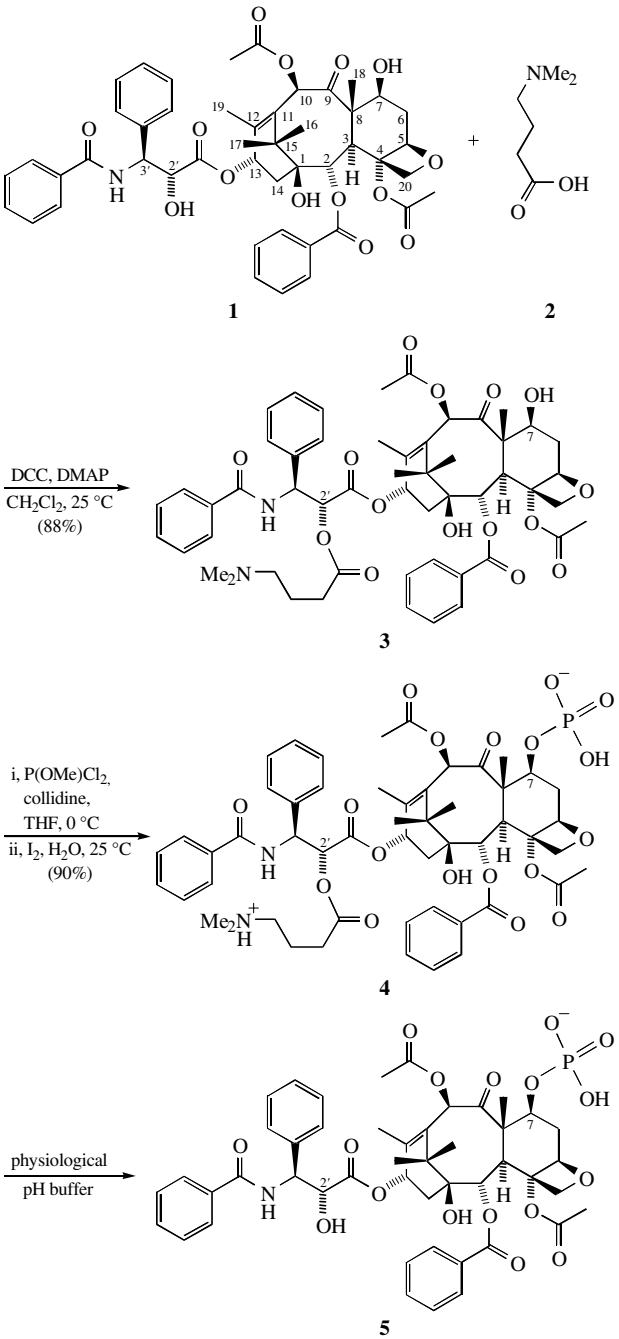
Paclitaxel **1** is one of the most promising anticancer agents;[1] it inhibits cell division and other interphase processes by stabilising microtubules.[2–6] This anticancer agent has an extremely low solubility in water.[4,5] Thus, enormous efforts have been focused on the modification of paclitaxel in order to create more water-soluble and, consequently, more easily formulated and delivered drugs.[7]

New paclitaxel derivatives could be designed to convert, in a predictable fashion, into the original active drug by either an enzymic mechanism[8–12] or simple hydrolysis initiated under physiological pH conditions.[13] Most accounts to date for paclitaxel **1** have been concerned with its esterifications at the C-2′[7,11,14–21] or C-7[11,22–25] hydroxyl group for improvement of the water solubility while the cytotoxic activity is maintained.[7,14–19,21–23,25]

In general, dephosphorylation occurs easier in cancer cells than in normal cells. Thus, chemotherapeutic agents possessing a phosphate or phosphoamidate unit would preferentially interact with the cancer cells.[26] Given these phenomena, we designed and synthesised new paclitaxel-containing aminoester phosphate **4** (Scheme 1) and phosphoamidate **9** (Scheme 2)[27] with higher water solubility. These new propaclitaxel analogues were found to possess anti-leukemic activity slightly greater than that of paclitaxel.

To synthesise 2′-[4-(*N*,*N*-dimethylammonium)butyryl]paclitaxel 7-phosphate **4**, we condensed paclitaxel **1** with 4-(*N*,*N*-dimethylamino)butyric acid **2** in the presence of dicyclohexylcarbodiimide (DCC) and a catalytic amount of (dimethylamino)pyridine (DMAP) in CH_2Cl_2 at 25 °C (Scheme 1).† Corresponding amino ester **3** was obtained in 88% yield. The reaction of **3** with $P(OMe)Cl_2$ in the presence of collidine in THF at 0 °C and then with I_2 and water at 25 °C produced the target compound, ammonium ester phosphate **4**, in 90% yield.[27] Compound **4** existed in its zwitterionic form.

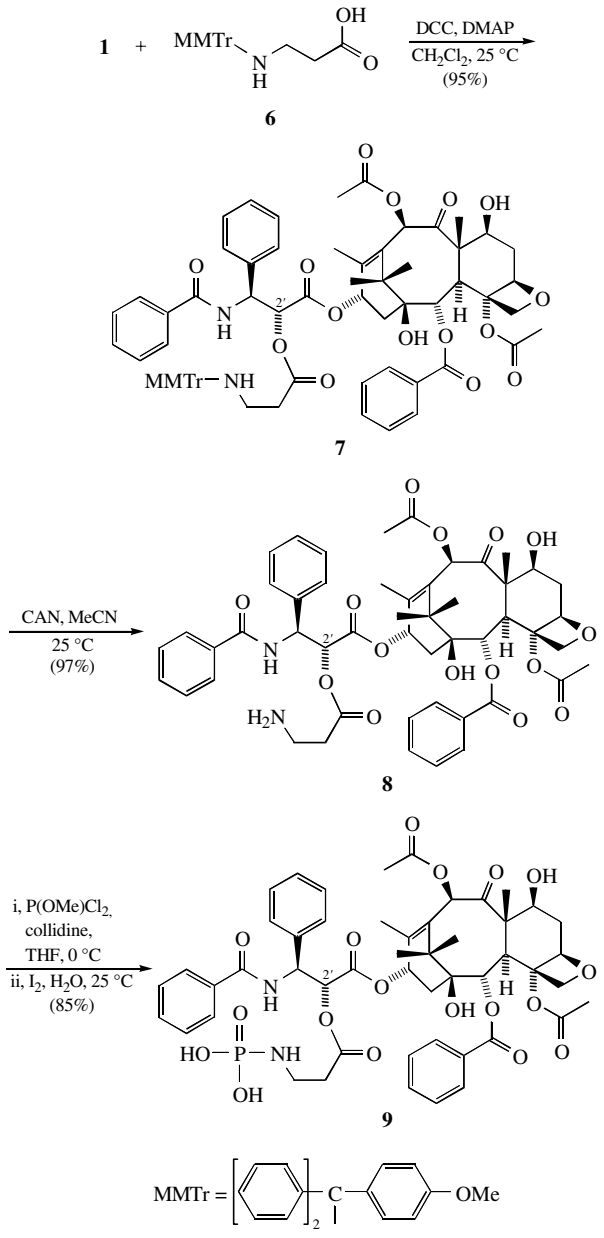
For the preparation of 2′-[3-(phosphoamido)propionyl]paclitaxel **9**, we treated paclitaxel **1** with 3-(*N*-monomethoxytritylamino)propionic acid **6** in the presence of DCC and DMAP in CH_2Cl_2 at 25 °C to give monomethoxytritylated amino ester **7** in 95% yield (Scheme 2).‡ Compound **7** in wet acetonitrile was treated with a catalytic amount of ceric ammonium nitrate (CAN) at 25 °C to afford detritylated amino ester **8** in 97% yield.[28] The reaction of **8** with $P(OMe)Cl_2$ and collidine in THF and then with I_2 and water produced desired phosphoamidate **9** in 85% yield.[27]



Scheme 1

† The structure of compound **3** was confirmed by the ^{1}H NMR ($CDCl_3$, 300 MHz) spectrum, which showed characteristic peaks at δ 2.17 (s, 6H, 2NMe) and 5.50 (d, 1H, 2′-H, *J* 3.2 Hz). Compound **4** showed characteristic peaks in ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 5.07 (dd, 1H, 7-H, *J* 10.7, 6.4 Hz) and 7.11 (br. d, 1H, NH).

‡ The structure of compound **7** was confirmed by the ^{1}H NMR ($CDCl_3$, 300 MHz) spectrum, which showed characteristic peaks at δ 3.75 (s, 3H, OMe) and 5.68 (d, 1H, 2′-H, *J* 7.1 Hz). Compound **8** showed a characteristic peak in ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 3.14 (br. s, 2H, NH_2). Compound **9** showed a characteristic peak in ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.03 (d, 1H, NH, *J* 7.6 Hz).



Scheme 2

The solubility (μmol dm^{-3}) in a phosphate buffer solution (0.10 M, pH 6.5) was 896 for **4**, 952 for **9** and 57.8 for paclitaxel **1**. Paclitaxel-containing aminoester phosphate **4** and phosphoamidate **9** were tested *in vitro* against HL-60 leukemic cells.[29,30] The IC_{50} values (μmol dm^{-3}) were 4.0×10^{-3} for **4**, 3.8×10^{-3} for **9** and 4.5×10^{-3} for **1**.

In comparison with paclitaxel **1**, new compounds **4** and **9** were found ~16 times more soluble in a phosphate buffer solution, and they exhibited comparable anti-leukemic activities *in vitro*. The free C(2′)–OH group is essential for the anticancer activity of paclitaxel.[27] Therefore, hydrolysis of the ester component in **4** and **9** under physiological pH conditions to give **5** and **1**, respectively, is responsible for the anti-leukemic activity of these new paclitaxel derivatives. On the other hand, the C(7)-phosphate functionality in **5** cannot be hydrolysed *in vitro* where phosphoesterases are not present. Thus, the C(7)-phosphate derivative of paclitaxel (*i.e.*, **5**) possesses anti-leukemic activity comparable with that of paclitaxel **1**.

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